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Paradigm for drug discovery employing encoded combinatorial libraries

(medicinal chemistry/isozyme selectivity/combinatorial chemistry)

JONATHAN J. BURBAUM*, MICHAEL H. J. OHLMEYER, JOHN C. READER, IAN HENDERSON, LAWRENCE W. DILLARD, GE LI, TROY L. RANDLE, NOLAN H. SIGAL, DANIEL CHELSKY, and JOHN J. BALDWIN

Departments of Biology and Chemistry, Pharmacopeia, Inc., 101 College Road East, Princeton, NJ 08540

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ABSTRACT **Very large combinatorial libraries of small molecules on solid supports can now be synthesized and each library element can be identified after synthesis by using chemical tags. These tag-encoded libraries are potentially useful in drug discovery, and, to test this utility directly, we have targeted carbonic anhydrase (carbonate dehydratase; carbonate hydro-lyase, EC 4.2.1.1) as a model. Two libraries consisting of a total of 7870 members were synthesized, and structure–activity relationships based on the structures predicted by the tags were derived. Subsequently, an active representative of each library was resynthesized {2-[*N*-(4-sulfamoylbenzoyl)-4′-aminocyclohexanespiro]-4-oxo-7-hydroxy-2,3-dihydrobenzopyran and [*N*-(4-sulfamoylbenzoyl)-L-leucyl]piperidine-3-carboxylic acid} and these compounds were shown to have nanomolar dissociation constants (15 and 4 nM, respectively). In addition, a focused sublibrary of 217 sulfamoylbenzamides was synthesized and revealed a clear, table structure–activity relationship describing isozyme-ective carbonic anhydrase inhibitors.**

The current national focus on health care reform has highlighted the continuing importance of developing more cost-effective methods for treating disease. Historically, the pharmaceutical industry has contributed to this effort by discovering and developing therapeutic molecules. The development of drug candidates is expensive (1, 2), and preclinical research toward the discovery and development of lead structures contributes significantly to this expense. For example, in 1991, the costs of medicinal chemistry and biological testing prior to safety assessment amounted to nearly 30% of domestic research costs (3). Apart from the economics of drug discovery, classical medicinal chemistry cannot efficiently address the plethora of new biochemical targets suggested by recent discoveries in molecular genetics, and lead structures from natural sources are often too complex for the cost-effective synthesis of analogs. Hence, targets for therapeutic intervention cannot be rapidly and efficiently exploited.

Combinatorial chemical synthesis applied to drug discovery promises to improve the productivity of medicinal chemistry both by significantly increasing the number of molecules available for testing and by providing facile routes toward synthetic analogs of active molecules. Many strategies for the generation of chemical diversity have been proposed (4–7). With a single exception (4), these approaches have been confined to the synthesis of flexible oligomeric ligands of relatively high molecular weight (e.g., peptides and oligonucleotides) which tend to be poor therapeutic agents, in part because of their lack of availability and stability *in vivo*.

For drug discovery, small-molecule libraries containing diverse functionality in a variety of molecular scaffolds possess the greatest utility. To construct such structurally diverse libraries while allowing the assignment of a structure to each member, we use a set of chemically stable molecular tags during solid-phase synthesis on polymeric beads (8). These tags can be used to unambiguously encode each bead with the synthetic scheme for its library member and are sufficiently robust to allow a wide range of chemical reactions during ligand construction.

A drug discovery effort based on such combinatorial libraries must pass several tests to be successful. First, the library must be diverse enough to permit the identification of a small subset of active molecules among many less active or inactive ones. Second, the most promising candidates in the library should suggest a testable structure–activity relationship. Third, since selectivity for a particular target is often important, strategies for the analysis of libraries containing a significant fraction of active compounds should result in optimization of selectivity as well as activity.

To explore the capabilities of encoded combinatorial libraries in a small-molecule drug discovery effort directed toward lead identification and optimization, we chose carbonic anhydrase (carbonate dehydratase; carbonate hydro-lyase, EC 4.2.1.1) as a therapeutically significant model, since inhibitors of carbonic anhydrase are known to be useful in ameliorating the symptoms of glaucoma. The inhibition of carbonic anhydrase by compounds containing primary sulfamoyl groups ($-SO_2NH_2$) has been well-characterized, both pharmacologically (9–11) and structurally (12–15). This allowed the design of test libraries that were predisposed toward showing carbonic anhydrase inhibition without explicitly including known inhibitors. Finally, since a number of different forms of the enzyme have been characterized, we were able to perform experiments to probe isoform selectivity.

MATERIALS AND METHODS

Materials. TentaGel S-NH_2 resin (particle size, 130 μm) was obtained from Rapp Polymere (Tubingen, Germany). The photocleavable linker was synthesized as described (16). Carbonic anhydrase was obtained from Sigma. White 96-well polystyrene plates were obtained from Dynatech.

Library Synthesis and Analysis. Libraries were synthesized by the split synthesis protocol, and each bead thus contains one member of the library (17–19). All members were affixed via a photocleavable *o*-nitrobenzyl linker. To preserve the synthetic history of each bead, each library was indexed in a binary fashion using electrophoric tags. These tags were attached by using $[(CF_3CO_2)_2Rh]_2$-activated carbene insertion as described (20). After identification and isolation of beads, the tags were analyzed by gas chromatography (8).

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Abbreviations: DNSA, dansylamide; hCA(I) and hCA(II), human carbonic anhydrase isozymes I and II.

*To whom reprint requests should be addressed.

Synthesis of Dihydrobenzopyran Library. First, three dihydroxyacetophenones (2-acetyl-1,4-dihydroxybenzene, 2-acetyl-1,5-dihydroxybenzene, and 2-acetyl-6-methyl-1,5-dihydroxybenzene) were coupled using Mitsunobu conditions (triphenylphosphine/diethylazodicarboxylate in tetrahydrofuran at ambient temperature) to the *o*-nitrobenzyl linker, which was then coupled to the beads by carbodiimide coupling [di(isopropylcarbodiimide)/hydroxybenzotriazole in dimethylformamide]. These intermediates were cyclized on the resin with a set of seven ketones, four of which contained a protected amine functionality. Modification of the amine functionality (when present) with 31 different headgroups under standard conditions produced a library of ketones containing 381 members. The ketones were then reduced with $NaBH_4$, converted into the corresponding dithiolanes with ethane-1,2-dithiol, or left unaltered to produce a library of 1143 distinct compounds.

Synthesis of Acylpiperidine Library. Library elements were coupled as either a carboxylic ester or a carbonate ester of an *o*-nitrobenzyl alcohol. First, five piperidines (2-hydroxymethyl-, 3-hydroxymethyl-, 4-hydroxy-, 4-carboxylic-, and 3-carboxylic-), and two linear moieties (5-amino-3-oxopentanol and 6-aminohexanoic acid) were attached to this linker either by phosgene coupling in solution (for the alcohols) or directly to the resin (for the acids). Next, 31 amino acids, including 28 examples of both D and L isomers of the 20 commonly occurring α-amino acids, and 3 nonproteinogenic amino acids (piperidine-4-carboxylic acid, piperidine-3-carboxylic acid, and 6-aminohexanoic acid) were reacted with the free amine. Finally, 31 reagents were linked as carboxamides, carbamates, sulfonamides, and ureas, including three primary sulfonamides (4-sulfamoylbenzamido, 4-chloro-5-sulfamoylbenzamido, and 2,4-dichloro-5-sulfamoylbenzamido).

Library Analysis for Carbonic Anhydrase Inhibition. Beads were distributed into 96-well microtiter plates. Compounds were detached from the beads by UV irradiation (350 nm) and then transferred to assay plates. An assay solution (50 μl) consisting of 0.1 M phosphate buffer (pH 7.4) containing bovine carbonic anhydrase (0.3 μM) and dansylamide (DNSA; 0.6 μM) was added to each well. Fluorescence values (λ_{ex}, 274 nm; λ_{em}, 454 nm) were measured with a Perkin–Elmer model LS 50B spectrofluorimeter equipped with a microtiter plate reader accessory and were normalized (uninhibited, 100; empty, 0). Assay solutions from the initial screen identified as active were transferred to a second fluorescence plate and 5 μl of 2 mM DNSA in dimethyl sulfoxide was added to each well to increase [DNSA] ≈600-fold *in situ*. Under these conditions, chlorothiazide ($K_d \approx 75$ nM) was displaced, while acetazolamide ($K_d \approx 7.5$ nM) was not, suggesting that more potent inhibitors were less sensitive to displacement by elevated [DNSA].

Selectivity Analysis. Aliquots (one-third) from the same bead eluate were assayed against each isozyme. Concentrations of the two isozymes were matched using $\varepsilon^{1\%}_{280}$ values from the literature (21), and the [DNSA] was varied according to the literature values for K_d (DNSA) of the isozymes (22).

RESULTS AND DISCUSSION

Two model libraries for drug discovery—a 1143-member dihydrobenzopyran library and a 6727-member acylpiperidine library—were designed. The first (dihydrobenzopyran) library (Fig. 1*A*) was designed to include 4-sulfamoylbenzoyl amides that were expected to confer carbonic anhydrase activity. The second (acylpiperidine) library (Fig. 1*B*) was designed around a core aminocarboxamide derived from 31 amino acids, including nonproteinogenic amino acids and both the D and L configurations of several naturally occurring α-amino acids. The carboxamide was formed from the carboxylic acid with seven amines including four substituted piperidines. Three different sulfamoylbenzoyl groups were incorporated at the final step to confer carbonic anhydrase activity.

Activity of library members against bovine carbonic anhydrase was assessed by using a fluorescence-based ligand-displacement assay (9). Because each synthetic library contained >1000 beads per member, we characterized the activity of the library statistically to optimize our screening conditions. The dihydrobenzopyran library was determined to contain ≈1.4% actives, identified as those wells where the fluorescence decreased by $>5\sigma$. Similarly, in the acylpiperidine library, we determined that the samples contained ≈6% actives. These observations were consistent with the percentage of primary sulfonamides incorporated in the two libraries, which was predicted to be 3% and 10% for the dihydrobenzopyran and acylpiperidine libraries, respectively.

Over 2300 beads from the dihydrobenzopyran library were then assayed singly and 33 individual beads were chosen for decoding. Thus, 2.0 library equivalents were assayed, where one library equivalent is defined as the number of beads equal to the number of distinct library members. The probability that a given compound was not assayed is approximated as follows: In a library of N total beads $\gg M$, the total number of library members, when S beads have been assayed, the probability that a given member has not been chosen is given by

$$P_s(0) \approx \left[1 - \frac{1}{M}\right]^S, \qquad [1]$$

which is approximately $(e^{-1})^{S/M}$ for large S. The quantity S/M is L, the number of library equivalents that have been assayed, so $P_s(0) \approx e^{-L}$. For the case at hand, the fraction of the library assayed at least once is $(1 - e^{-2})$ or 86%.

The larger acylpiperidine library was pooled at the final reaction step into two smaller portions to permit a more convergent assay strategy. These pools consisted of 3472 and 3255 members, the larger portion containing all the primary sulfonamides. For the smaller portion, ≈5 library equivalents (≈17,000 beads) were assayed at 10 beads per well, and no actives were identified. Thus, in Eq. 1, $L \approx 5$ and $P_s(0)$ is <0.01. Hence, >99% of the members were assayed at least once, and none were found to be active under the conditions described.

We therefore focused our continued efforts on the portion of the acylpiperidine library that contained primary sulfonamides. Here, half the eluates from a total of 4320 single beads (1.3 library equivalents) were assayed individually, and >300 actives were identified (Fig. 2*A*). To estimate the relative potency of the active inhibitors, the stringency of the assay was increased by raising the concentration of the competitor, DNSA. However, the associated background fluorescence made the fluorescence decrease caused by inhibitors less easily detected. Potent actives were therefore judged as differing by $>3\sigma$ compared to the median active compound. Eighteen high-affinity actives were thereby identified and decoded (Fig. 2*B*).

As anticipated, we found that carbonic anhydrase inhibitors from either library exclusively contained the sulfamoyl group ($-SO_2NH_2$). From the actives, we chose two synthetic targets, one from the dihydrobenzopyran library (compound **1**) and one of the more potent actives from the acylpiperidine library (compound **2**). These compounds were resynthesized, their structures were confirmed spectroscopically, and their K_d values vs. carbonic anhydrase were determined experimentally (Table 1). The low nanomolar values for compounds **1** and **2** support the use of tag-derived information for structure–activity assessments in the libraries we report.

Significantly, no structures containing a 4-chloro-5-sulfamoylbenzoyl moiety were found among the tag-predicted structures for potent actives in the acylpiperidine library. Furthermore, while the other two sulfamoylbenzamides were

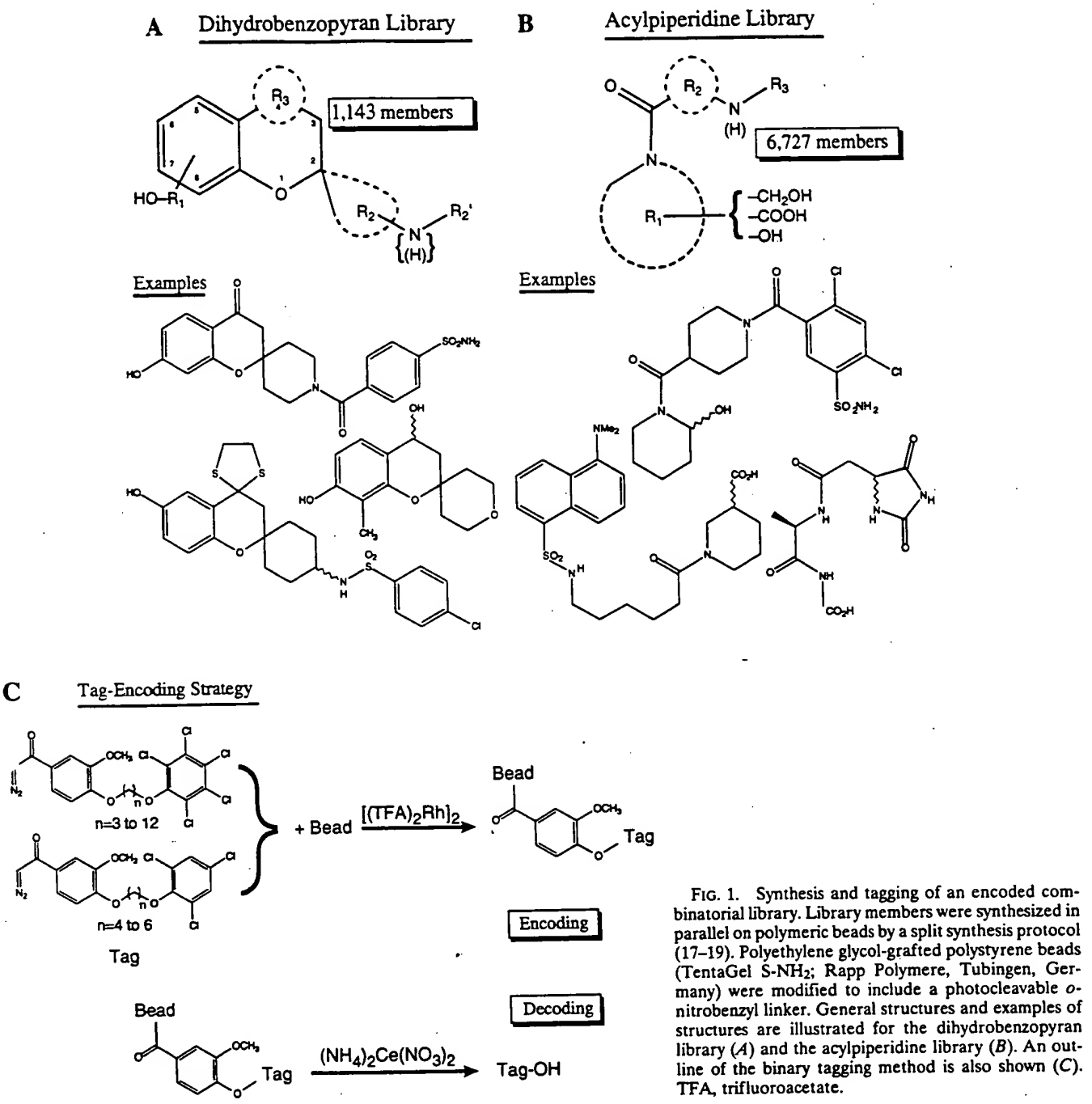


FIG. 1. Synthesis and tagging of an encoded combinatorial library. Library members were synthesized in parallel on polymeric beads by a split synthesis protocol (17–19). Polyethylene glycol-grafted polystyrene beads (TentaGel S-NH_2; Rapp Polymere, Tubingen, Germany) were modified to include a photocleavable *o*-nitrobenzyl linker. General structures and examples of structures are illustrated for the dihydrobenzopyran library (*A*) and the acylpiperidine library (*B*). An outline of the binary tagging method is also shown (*C*). TFA, trifluoroacetate.

well-represented, they showed markedly different structure–activity relationships. For example, the 4-sulfamoylbenzamides appear to prefer a hydrophobic R_2 group of the L configuration, while the 2,4-dichloro-5-sulfamoylbenzamides appear to tolerate more diversity at R_2, including D-alanine. Thus, to test the validity of this observation, library-specific homologs of the acylpiperidine (compound 2) were synthesized (Table 1, compounds 3 and 4). The 150-fold increase in K_d seen upon substitution further validates the ability of the screen to select active compounds and supports the divergent structure–activity relationships observed with the various sulfonamide headgroups.

In developing a class of compounds for use as therapeutic agents, increased affinity is but one criterion for optimization. Isozyme selectivity is often an additional critical property required to minimize side effects. The selectivity of a large library is difficult to optimize convergently (i.e., with >1 element per assay), because such assays must measure the absence of activity against a particular target or relative levels of activity vs. multiple targets. As the percentage of actives increases, the probability that the observed affinities are the property of multiple elements increases. Thus, approaches taken to identify high-affinity peptides in a convergent fashion (e.g., see ref. 6) become impractical with selectivity, and focused sublibraries assayed singly become pertinent. To evaluate the potential for using encoded combinatorial libraries to optimize selectivity, a 217-member sublibrary (consisting of the 4-sulfamoylbenzamides from the larger acylpiperidine library) was prepared and evaluated against human carbonic anhydrase isozymes I and II [CA(I) and CA(II)].

To isolate selectivity as a variable, two factors were normalized. First, equal inhibitor concentrations were exposed to each isozyme by taking aliquots from the same bead eluate. Thus, one-third of a complete eluate was assayed vs. human CA(I), and one-third was assayed vs. CA(II). Second, the concentrations of the two isozymes were matched, but the

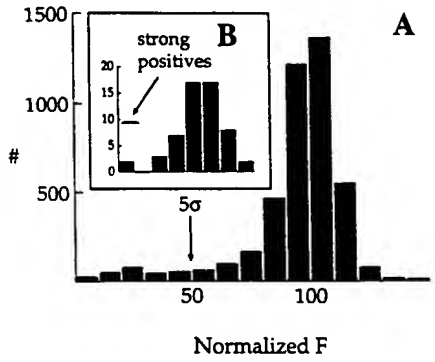


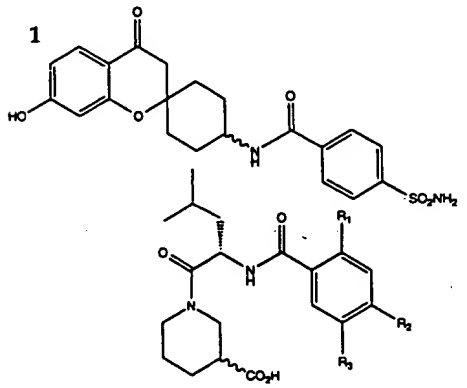
FIG. 2. Histogram analysis of combinatorial libraries. (*A*) Low-stringency library analysis. Half the eluates from a total of 4320 single beads (1.2 library equivalents) were assayed individually, and >300 actives (5σ) were identified. For the dihydrobenzopyran library, >2300 members (2.0 library equivalents) were assayed singly and 33 individual beads were identified and decoded. (*B*) Reanalysis of positives from *A* at higher stringency. To increase the stringency of the assay, the concentration of DNSA was increased ≈600-fold *in situ*, and 18 potent actives were selected for decoding.

[DNSA] was varied according to the literature values for K_{DNSA} of the isozymes. These requirements derive from the binding equation, given relative to isozyme x

$$F_x \propto [E_x\text{·DNSA}] = \frac{[E]_{tot,x}}{1 + \frac{K_{DNSA,x}}{[DNSA]_x}\left(1 + \frac{[I]}{K_{i,x}}\right)}. \quad [2]$$

Thus, when E_{tot} is equal, the ratio $[DNSA]/K_{DNSA}$ is equal, and [I] is equal, the only variable that can affect F is the dissociation constant for the inhibitor, K_i. To identify samples for which there is a significant difference in K_i for the two isozymes, the normalized fluorescence values were analyzed graphically (Fig. 3).

Table 1. Analysis of compounds and validation of tag-derived structure–activity relationships

Compound				$-K_d$[bCA(II)], nM
1				15
	R_1	R_2	R_3	
2	H	SO_2NH_2	H	4
3	H	Cl	SO_2NH_2	700
4	Cl	Cl	SO_2NH_2	660

Structures of compounds 1–4 were confirmed analytically by ^1H NMR spectroscopy and mass spectrometry. K_d values were measured as described (7) with EXCEL software to solve the multiple equilibria numerically. b, Bovine.

Fig. 3*A* shows a selectivity plot comparing the normalized fluorescence values for the two enzymes. In this figure, region A contains compounds that show potency vs. both isozymes, while region B contains compounds that are less selective for CA(II) than the median, and region C contains compounds that are more selective than the median. Sulfonamides that are not particularly active against either isozyme are found in region D. Beads from each region were decoded, and examples of the structures predicted by these codes are shown in Fig. 3*B*. The potent compound that was identified in screening against bovine carbonic anhydrase (Table 1, compound 2) was iden-

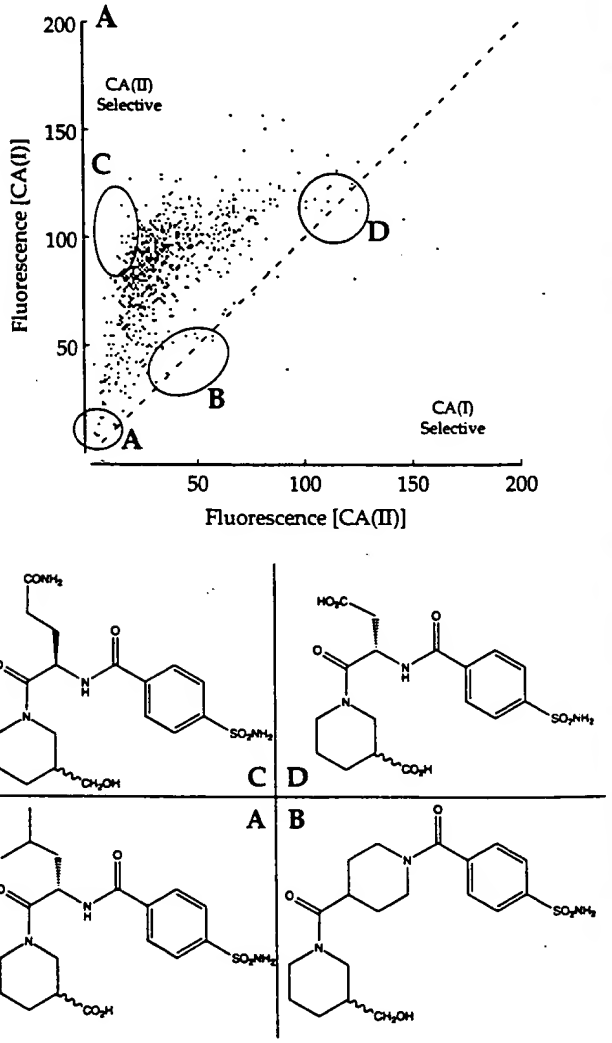


FIG. 3. Selectivity assay of a highly active sublibrary. (*A*) Inhibitors were analyzed independently for each isozyme as in Fig. 2 and then plotted. The observation that the majority of the library members are found above the diagonal suggests that the selectivity for hCA(II) may be a property of this class of compounds rather than a characteristic of a given member. (*B*) From tag-derived relationships, there are clear trends associated with the different regions, and some examples of compounds are shown. The characteristics of the potent inhibitors (region A) are described above, and the potent compound that was identified in assays against bovine carbonic anhydrase (Table 1, compound 2) was found here. Region B (relatively nonselective) shows a preference for cyclic imino acids in the central region, which may reflect differential hydrogen bonding by the carboxamido nitrogen of the sulfamoylbenzamide (13) or to a positive discrimination by the ring. Region C [relatively hCA(II) selective] shows a preponderance of polar side chains, as well as a relative lack of sensitivity toward the C^α configuration. Region D (low activity) contains more polar compounds but, notably, one D-leucyl side chain that complements the strong preference for hydrophobic groups of the L stereoconfiguration noted above.

tified again in region A. Analysis of the selectivity of this compound showed that it has a 3-fold selectivity for human (h) CA(II) {K_d[hCA(II)] = 11 nM; K_d[hCA(I)] = 33 nM}, but this would not be apparent from the plot because of its ively high affinity. Since the other compounds in this small sublibrary are also analogs of 4-sulfamoylbenzamide, the observation that most library members are found above the diagonal suggests that the selectivity observed for compound 2 may be a property of this class of compounds rather than a characteristic of this particular member. Nevertheless, there are clear trends associated with the different regions. Region B (relatively nonselective) shows a preference for cyclic imino acids in the central region, which may reflect differential hydrogen bonding by the carboxamido nitrogen of the sulfamoylbenzamide (13), or to a positive discrimination by the ring. Region C [relatively hCA(II) selective] shows a preponderance of polar side chains as well as a relative lack of sensitivity toward the C^{α} configuration. Region D (low activity) contains more polar compounds but, notably, one D-leucyl side chain that complements the strong preference for hydrophobic groups of the L stereoconfiguration noted above. Together, the trends derived from library analysis suggest that more selective compounds could be synthesized, either as single compounds or as appropriately designed focused libraries.

Conclusions. We have synthesized encoded libraries containing 10^3–10^4 small molecules by using diverse chemistries. Active carbonic anhydrase inhibitors within these libraries have been identified and optimized by using directed sublibraries based on the original lead and the chemistry developed for its synthesis. This application of combinatorial libraries for drug discovery addresses two pressing needs of the pharmaceutical industry. First, small-molecule leads will become more readily available as the size and diversity of synthetic libraries reases. This, in turn, will accelerate the discovery of compounds active against a variety of therapeutic targets, especially those that emerge from the explosion in sequence information arising from the human genome project. Second, synthetic chemists involved in drug discovery will be more efficiently engaged to solve sophisticated structure–activity problems. The success of the combinatorial approach emphasizes the power of techniques for the rapid synthesis and evaluation of large numbers of possible structures and anticipates a new age of drug discovery and optimization. In the future, analysis of combinatorial libraries may be used to direct research efforts toward productive avenues by suggesting the synthesis either of a handful of pertinent test compounds or of combinatorial libraries based on relevant structural types. Indeed, such a combinatorial approach may be productively applied not just in pharmaceutical discovery, but in any area where optimization of molecular properties is necessary.

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1. Vagelos, P. R. (1991) *Science* **252,** 1080–1084.
2. Kirschner, M. W., Marincola, E. & Teisberg, E. O. (1994) *Science* **266,** 49–51.
3. Pharmaceutical Research and Manufacturers of America (1993) *PMA Annual Survey Report: Trends in U. S. Pharmaceutical Sales and R & D* (Pharmaceut. Manuf. Am., Washington, DC), p. 29.
4. Bunin, B. A. & Ellman, J. A. (1992) *J. Am. Chem. Soc.* **114,** 10997–10998.
5. Needels, M. C., Jones, D. G., Tate, E. H., Heinkel, G. L., Kochersperger, L. M., Dower, W. J., Barrett, R. W. & Gallop, M. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10700–10704.
6. Houghten, R. A., Pinilla, C., Blondelle, S. E., Appel, J. R., Dooley, C. T. & Cuervo, J. H. (1991) *Nature (London)* **354,** 84–86.
7. Simon, R. J., Kania, R. S., Zuckermann, R. N., Huebner, V. D., Jewell, D. A., Banville, S., Ng, S., Wang, L., Rosenberg, S., Marlowe, C. K., Spellmeyer, D. C., Tan, R., Frankel, A. D., Santi, D. V., Cohen, F. E. & Bartlett, P. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9367–9371.
8. Ohlmeyer, M. H. J., Swanson, R. N., Dillard, L. W., Reader, J. C., Asouline, G., Kobayashi, R., Wigler, M. & Still, W. C. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10922–10926.
9. Ponticello, G. S., Freedman, M. B., Habecker, C. N., Lyle, P. A., Schwam, H., Varga, S. L., Christy, M. E., Randall, W. C. & Baldwin, J. J. (1987) *J. Med. Chem.* **30,** 591–597.
10. Hartman, G. D., Halczenko, W., Smith, R. L., Sugrue, M. F., Mallorga, P. J., Michelson, S. R., Randall, W. C., Schwam, H. & Sondey, J. M. (1992) *J. Med. Chem.* **35,** 3822–3831.
11. Lo, Y. S., Nolan, J. C., Maren, T. H., Welstead, W. J., Jr., Gripshover, D. F. & Shamblee, D. A. (1992) *J. Med. Chem.* **35,** 4790–4794.
12. Jain, A., Whitesides, G. M., Alexander, R. S. & Christianson, D. W. (1994) *J. Med. Chem.* **37,** 2100–2105.
13. Cappalonga Bunn, A. M., Alexander, R. S. & Christianson, D. W. (1994) *J. Am. Chem. Soc.* **116,** 5063–5068.
14. Ericksson, A. E., Kylsten, P. M., Jones, T. A. & Liljas, A. (1988) *Proteins Struct. Funct. Genet.* **4,** 283–293.
15. Smith, G. M., Alexander, R. S., Christianson, D. W., McKeever, B. M., Ponticello, G. S., Springer, J. P., Randall, W. C., Baldwin, J. J. & Habecker, C. N. (1994) *Protein Sci.* **3,** 118–125.
16. Barany, G. & Albericio, F. (1985) *J. Am. Chem. Soc.* **107,** 4936–4942.
17. Furka, Á., Sebesteyén, F., Asgedom, M. & Dibó, G. (1991) *Int. J. Peptide Protein Res.* **37,** 487–493.
18. Sebesteyén, F., Dibó, G., Kovacs, A. & Furka, Á. (1993) *Bioorg. Med. Chem. Lett.* **3,** 413–418.
19. Lam, K. S., Salmon, S. E., Hersh, E. M., Hruby, V. J., Kazmierski, W. M. & Knapp, R. J. (1991) *Nature (London)* **354,** 82–84.
20. Nestler, H. P., Bartlett, P. A. & Still, W. C. (1994) *J. Org. Chem.* **59,** 4723–4724.
21. Nyman, P. O. & Lindskog, S. (1964) *Biochim. Biophys. Acta* **85,** 141–151.
22. Taylor, P. W., King, R. W. & Burgen, R. S. V. (1970) *Biochemistry* **9,** 2638–2645.